

EFFECTS OF ACCLIMATIZATION AND SEX ON RESPIRATION AND THERMAL RESISTANCE IN *TRIBOLIUM* (COLEOPTERA:TENEBRIONIDAE)¹

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Abstract

Some effects have been studied of prolonged exposure to nonoptimal temperatures on respiration and thermal resistance in the flour beetle *Tribolium confusum* Duval.

Adult beetles maintained at 18° C. and 38° C. for a number of months exhibited changes in the location and form of the temperature-respiration curve compared with that of control insects at 30° C. Insects from 18° C. showed increased survival ability at -3° C., but there was no respiratory compensation during acclimatization. Changes in the sign of respiration-weight regression in the experimental groups was probably related to differences in sensitivity to temperature between lighter and heavier individuals.

Control 30° males survived exposure to -3° C. better than corresponding females. This survival difference at the low temperature was eliminated by prolonged maintenance of the insects at 18° C. Insects from 18° C. possessed a higher water content and a lower dry weight than controls. A discussion is presented dealing with the influence of the ratio of active to inactive weight on respiration per unit weight.

The temperature-respiration curves were adequately expressed in the double-logarithmic form. The constants in this equation were used comparatively among the different experimental groups.

Introduction

Previous temperature history is one of the most important factors influencing the nature of the metabolic response of an insect to temperature (30). Acclimatization to cold occurs in insects, but the evidence for experimental heat acclimatization in insects is still not great.

Robinson (29) carried out studies of acclimatization to cold in various insects and showed that they can adapt. Mellanby (19), in studies of cold adaptation in various insect genera, showed that those from a warm environment were less cold-hardy than those from a cooler environment. Adjustment to a lower temperature, measured by activity, occurred in these insects within twenty hours. This same author (20) found in some species of Arctic insects that the stages normally exposed to cold for long periods were less immobilized by temperatures down to 0° C. than stages which were normally exposed to a warm environment. Whitney (36), working with May fly nymphs from ponds and streams, found that those from slow or still waters possessed greater heat tolerance than those from swift streams. He related the greater thermal adaptation to greater temperature fluctuations in the undisturbed environment. Walshe (35) found that larvae from streams below 15° C. were less resistant to high temperatures than larvae from still waters at 20° C. Baldwin (2) and Baldwin and House (3) increased the thermal

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resistance of sawfly larvae by thermal conditioning. Colhoun (9) acclimatized *Blatta* to cold, thermal conditioning being evidenced by a greater activity at low temperatures.

Agrell (1) investigated the influence of previous temperature history on a number of insects which had undergone thermal adaptation over a period of weeks. Insects were transferred from their normal environmental temperature to 5°, 18°, and 30° C. The respiration of each group was measured at 18° C. All experimental insects showed a quantitative change in gas consumption with changes in the previous temperature history. Agrell stated that insects may respond to temperature changes either with or without respiratory compensation.

Bullock (7) concluded that there are general indications of poor compensatory adjustment to temperature changes in insects. However, Dehnell and Segal (11) and others have shown that some insects exhibit respiratory adaptation at different temperatures.

The present work represents a portion of a study of various factors which affect the respiration and thermal resistance of *Tribolium confusum* Duval, the confused flour beetle (12). It was intended to establish first a temperature-respiration curve for male and female normal *T. confusum* reared at 30° C. These curves could be used as controls for comparison with curves obtained from insects with different previous temperature histories. A comparison between control insects and those with different previous temperature histories could also be made on the basis of survival at extreme temperatures.

A preliminary report on some of the results from these experiments has already been made (13).

Methods

Adult *T. confusum* were used in all the experiments. Cultures of this species have been maintained at McGill University for over 10 years. These cultures are descendants of original stock used by R. N. Chapman for his work on the biotic constants of this insect (8). The cultures were therefore quite uniform genetically. The food medium was whole-wheat flour with 3% ground wheatgerm added. The cultures were maintained at 30° C., the physiological optimum (14, 34), and at a relative humidity of 75%.

For sexing the insects, the method of Hope (16) was used. This method is based upon the pattern of the lines engraved on the elytra. Well in excess of 100 live *T. confusum* can be sexed in 1 hour using this technique.

Some insects were taken from the 30° control culture as 2-months-old adults and maintained in constant temperature cabinets at 18° C. and 38° C., relative humidity 75%. They were kept at these temperatures for approximately six months prior to their being used in experiments.

Respiration was measured over a series of temperatures with Barcroft respirometers. A single sample consisted of 10 insects of one sex. The sample was put into the appropriate respiration chamber of the respirometer with small strips of flour-paper (10). The cup, with the insects, was allowed

to stand overnight in the constant-temperature cabinet from which the insects had just been taken. This "waiting period" was found to be necessary in order to allow the insects to recover fully from handling effects. The following day the oxygen consumption was determined at the temperature to be investigated. The insects remained quiescent in the respiration chamber during the experiment. Readings were taken until the manometer deflection assumed a constant value. The sample of insects was then removed and weighed, and the oxygen consumption per milligram determined. Thus, one point was established on the temperature-respiration curve under investigation. Five such points were obtained at each experimental temperature for each sex.

Survival at -3°C . and $+40^{\circ}\text{C}$. was investigated for 30° control insects and insects maintained at 18°C . and 38°C . One-liter glass museum jars were used to contain the insects at the experimental temperatures. Samples of experimental and control insects were placed in the jars together with a small dish of a saturated salt solution to maintain the relative humidity as close to 75% as possible.

Control insects which had been reared and maintained at 30°C . will henceforth be referred to as 30° insects. The two experimental groups will be referred to as the 18° and the 38° insects.

Observations

A. Respiration

The values for oxygen consumption per unit weight for control 30° insects and for both 18° and 38° insects are given in Table I with 95% confidence limits. Only females were investigated in the 38° group. Each value for oxygen consumption represents the mean of five determinations. An overlapping of the confidence limits of two comparative values (i.e. male and female values at the same temperature, or values for the control and the experimental groups at the same temperature and of the same sex) is taken to indicate no significant difference.

TABLE I
MEAN VALUES FOR OXYGEN CONSUMPTION AT VARIOUS TEMPERATURES
($\text{MM.}^3/\text{MG.}/\text{HR.}$) FOR 30° CONTROL, 18° , AND 38° *T. confusum*, WITH 95%
CONFIDENCE LIMITS

T.	30°		18°		38°
	♂	♀	♂	♀	♀
5°	0.160 ± 0.073	0.184 ± 0.052			
10°	0.317 ± 0.023	0.338 ± 0.038	0.165 ± 0.060	0.211 ± 0.123	0.179 ± 0.050
18°	0.808 ± 0.197	1.08 ± 0.14	0.450 ± 0.056	0.703 ± 0.176	0.745 ± 0.133
26°	1.54 ± 0.31	1.70 ± 0.04			
34°	2.63 ± 0.67	2.77 ± 0.21	1.97 ± 0.71	2.29 ± 0.51	2.21 ± 0.57
40°	3.54 ± 0.43	3.31 ± 0.06	2.90 ± 0.72	3.24 ± 0.52	2.88 ± 1.18
44°	3.80 ± 0.78	4.29 ± 0.46	3.63 ± 0.50	4.02 ± 0.78	3.84 ± 0.47

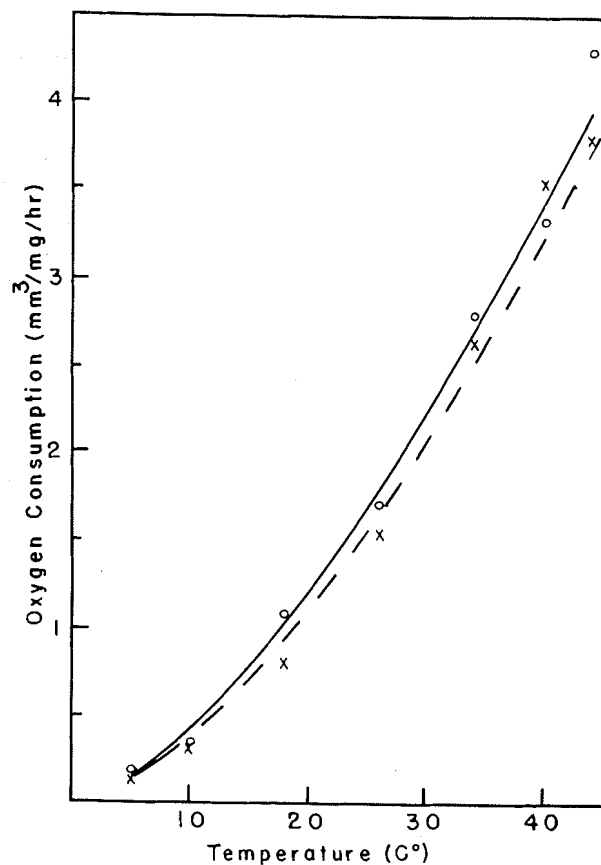


FIG. 1. T-R curves for male (broken) and female (solid) control 30° *T. confusum*. Each point near the lines indicates the mean of five respiration determinations (open circles for females).

The temperature-respiration curves for male and female 30° control *T. confusum* are illustrated in Fig. 1. Points near the curves indicate the mean values given in Table I. The lines of best fit were calculated by the method of least squares. A straight line resulted when the temperature-respiration data were plotted on a double-logarithmic grid. The following equation was therefore considered to be the most convenient way in which to express the data:

$$\log R = \log a + b \log T$$

where R represents the vertical axis, or oxygen consumption; T represents the horizontal axis, or temperature; a and b are constants. The equations of the lines of best fit for 30° males and females are:

$$\begin{aligned} \text{Males: } \log R &= -1.968 + 1.540 \log T \\ \text{or } R &= 0.011 T^{1.54} \end{aligned}$$

$$\begin{aligned} \text{Females: } \log R &= -1.855 + 1.490 \log T \\ \text{or } R &= 0.014 T^{1.49} \end{aligned}$$

An F test for goodness of fit of the data, with both variables in logarithmic form, indicated that 97.2% of the variation in $\log R$ in males was accounted for by regression of $\log R$ on $\log T$. Similarly, 98.2% of the $\log R$ variation in females was accounted for by regression on $\log T$. In both cases this may be considered a good fit of the log-log data to a straight line (33).

A comparison of the temperature-respiration curves of female 30°, 18°, and 38° *T. confusum* has already been reported (13). A table was presented in that report showing the values of the constants in the T-R curves together with 95% confidence limits. Table II in the present work includes data from that report and is extended to include the constants for males. Fig. 2 has been taken from the previous work. The temperature-respiration curves of female 30°, 18°, and 38° *T. confusum* are compared in semilogarithmic form, illustrating changes in Q_{10} over the temperature range. The Q_{10} is reflected by the slope of the tangents to the curves.

TABLE II
VALUES FOR T-R EQUATION CONSTANTS, WITH 95% CONFIDENCE LIMITS AND
RANGES, FOR 30° CONTROL, 18°, AND 38° *T. confusum*

Group	Sex	log a		b	
		Constant	Range (a)	Constant	Range (b)
18°	Males	-2.959 ± 0.199	0.000694-0.00174	2.124 ± 0.169	1.95-2.29
18°	Females	-2.707 ± 0.204	0.00123 -0.00314	2.008 ± 0.174	1.83-2.18
30°	Males	-1.968 ± 0.125	0.00808 -0.0143	1.540 ± 0.092	1.45-1.63
30°	Females	-1.855 ± 0.100	0.0111 -0.0175	1.490 ± 0.072	1.42-1.56
38°	Females	-2.717 ± 0.151	0.00136 -0.00271	2.002 ± 0.128	1.87-2.13

Calculation of the 95% confidence limit ranges for the constants in Table II shows that corresponding values for males and females do not differ significantly in either the 30° control group or the 18° group. Comparison of the constants for the same sex between the control and the experimental groups reveals significant differences. The values for a in both the 18° and the 38° groups are lower than the a value for the corresponding sex in the 30° controls. The values for b in the experimental groups are greater than that for the corresponding sex in the 30° controls.

Although the curve for males is depressed below that for females in Fig. 1, comparison of the values for oxygen consumption at any experimental temperature along the curves reveals no significant difference.

In Fig. 2 the T-R curves for 18° and 38° females are depressed below that for 30° females. On the basis of comparison of the constants a in the curve equations this depression is statistically significant. The significantly greater b in both the 18° and 38° curves compared with control curves is reflected in the greater curvature of the former. Thus, at any given temperature along the curve the Q_{10} in the 18° and 38° groups is greater than that of 30° controls.

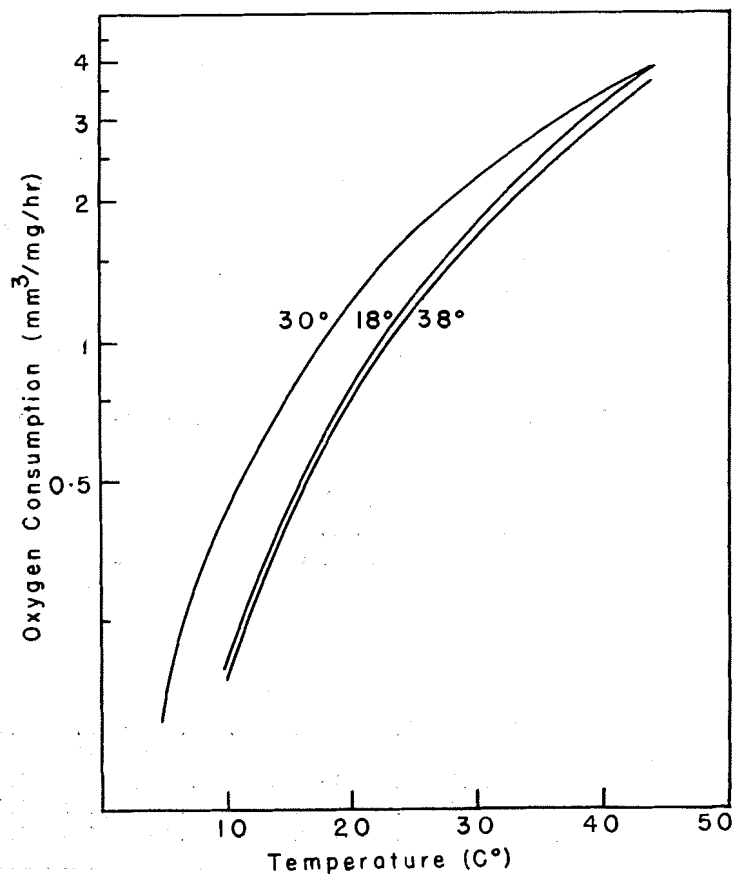


FIG. 2. Semilogarithmic plot of T-R curves of female *T. confusum* from 18° C., 30° C., and 38° C.

B. Survival at Extreme Temperatures

The results of exposure of 30° control, 18°, and 38° *T. confusum* to +40° C. for various times are given in Table III. Males and females are not treated separately, since no sex difference in survival ability was found at the high temperature. The LD₅₀ 95% confidence limits for each of the three groups exposed to 40° C. are given in Table IV.

Tables III and IV show that survival at 40° C. in the 18° insects was greater than that of 30° controls. Survival in the 38° group was less, compared with that of the controls. The statement of significance is based upon non-overlapping of the LD₅₀ 95% limits.

A sample of 30° control insects in each of the two experiments wherein 38° insects were exposed to 40° (4 and 5 days respectively) was used in a direct Chi² comparison with the latter to test for a difference in survival. In both the 4 and 5 day experiments the survival of the 38° insects was significantly lower than that of the corresponding controls. This is in agreement with the difference between the LD₅₀ values of the two groups.

A χ^2 test was made to compare two groups of 18° and 30° control insects kept for 4 days at 40° C. A significant difference in survival was apparent between 18° insects and 30° controls after 4 days at 40° C. There was a greater survival in the former group. This is in agreement with the difference between the two LD₅₀ values.

TABLE III

PER CENT SURVIVAL OF 30° CONTROL, 18°, AND 38° *T. confusum* DURING EXPOSURE TO +40° C. VALUES FOR DIFFERENT TESTS ARE GIVEN, WITH MEAN PER CENT SURVIVAL IN PARENTHESES; $n = 50$ IN EACH TEST

Exposure time, days	Per cent survival		
	30°	18°	38°
4	48		
	54	(49.3)	62
	46	96	(79)
4.8	38		24
	28		
5	34	(34)	76
	36		
	38		10
6	16	18	

TABLE IV

NINETY-FIVE PER CENT CONFIDENCE LIMITS FOR LD₅₀ VALUES FOR THREE TEMPERATURE GROUPS EXPOSED TO -3° C. AND +40° C.

Group	Sex	-3° C.	+40° C.
		LD ₅₀ limits, hr.	LD ₅₀ limits, days
30°	Male	42.61- 46.53	3.72-4.34
30°	Female	27.53- 38.98	
18°	Male	86.28-107.26	5.09-5.61
18°	Female	81.13- 96.39	
38°	Male	31.75- 50.40	0.81-1.66
38°	Female	22.57- 33.60	

The mortality trends in both sexes of 30°, 18°, and 38° *T. confusum* exposed to -3° C. are indicated in Fig. 3. Males and females are treated separately. Percentage mortality values were converted to probits and plotted against time at the experimental temperature. The intersection point of the line at the probit value 5.0 represents the LD₅₀. Points near the lines indicate mortality values based upon a mixed-sexes sample of 50 insects at each exposure time.

The values for the LD₅₀ were calculated by fitting a straight line to the mortality percentages converted to probits and solving the equation of the line

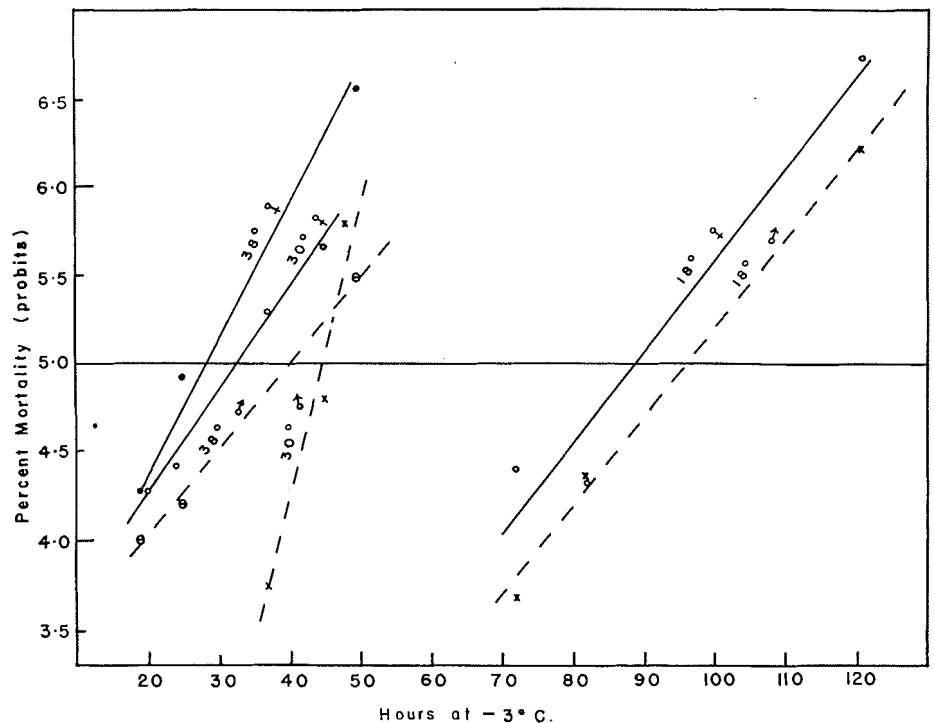


FIG. 3. Mortality in both sexes of *T. confusum* from three different temperatures after exposure to -3°C . Points near the lines indicate mean values for each sample.

for x (time) with y equal to 5.0. Confidence limits were placed about the LD_{50} values by the method of Litchfield and Wilcoxon (18). The 95% confidence limits for the LD_{50} values are given in Table IV.

The survival of male and female 30° controls after 24, 45, and 48 hours at -3°C . were compared by means of a Chi^2 test to determine whether a survival difference existed between the two sexes. The Chi^2 diagram is shown in Table V. The calculated Chi^2 is 4.4, while the chance value for Chi^2 is 3.8 (d.f. 1).

The Chi^2 test shows a significant difference in the survived-dead ratio between 30° control males and females at -3°C . The survival of males is greater, under the conditions of the experiment. This is in agreement with the smaller LD_{50} in 30° females shown in Table IV.

Both sexes of *T. confusum* kept for some time at 18°C . showed a significantly increased survival ability when compared with that of 30° controls (Table IV). In addition, prolonged exposure to 18°C . eliminated the sex difference in survival at -3°C . This is revealed by comparison of the two LD_{50} confidence limits.

Prolonged exposure of *T. confusum* to 38°C . did not significantly alter the LD_{50} at -3°C . compared with the same sex in the 30° control group. However, as was the case in the 18° insects, no sex difference in survival ability at the low temperature was apparent in the 38° insects.

TABLE V
NUMBER OF SURVIVING AND DEAD 30° CONTROL *T. confusum* AFTER
24, 45, AND 48 HOURS AT -3° C. THE TOTAL NUMBER OF INSECTS
AT EACH EXPOSURE TIME IS 50

	Survived	Dead	Σ
Males	26	3	88
	15	11	
	7	26	
	48	40	
Females	15	6	62
	6	18	
	2	15	
	23	39	
Σ	71	79	150

C. Early Effects of Nonoptimal Temperatures

Some experiments were carried out to determine the nature of the changes in oxygen consumption during the first few days of continual exposure of 30° control *T. confusum* to 18° C. and 38° C., relative humidity 75%. The oxygen consumption of a sample of 10 insects was first measured at 30° C., the control temperature. The insects were then transferred to either the high or low experimental temperature in the respirometer chamber. Further respiration determinations were made at daily intervals at the experimental temperature.

The results of the above experiments are shown in Figs. 4 and 5. Fig. 4 shows the change in the respiration of both sexes during exposure to 18° C. Fig. 5 illustrates the changes which occurred during exposure to 38° C. Oxygen consumption is expressed in cubic millimeters per insect per hour.

The respiration-time curves in Fig. 4 show that the oxygen consumption reacted passively to the lowered temperature, showing no respiratory compensation.

In Fig. 5 the oxygen consumption of both sexes increased immediately following exposure to 38° C. This increase corresponds to the change in respiration level along the temperature-respiration curve of control insects (Fig. 1). In males the oxygen consumption rose to a maximum 1 day after exposure to 38° C. In females the maximum respiration was reached in 2 days. The oxygen consumption fell beyond the maximum in both sexes. In males the "overshooting" of the respiration lasted 2 days. The respiration in males returned to a final level which was higher than that immediately following exposure to 38° C. In females the period of "overshooting" lasted 3 days and the respiration returned to the level of that immediately following initial exposure to 38° C.

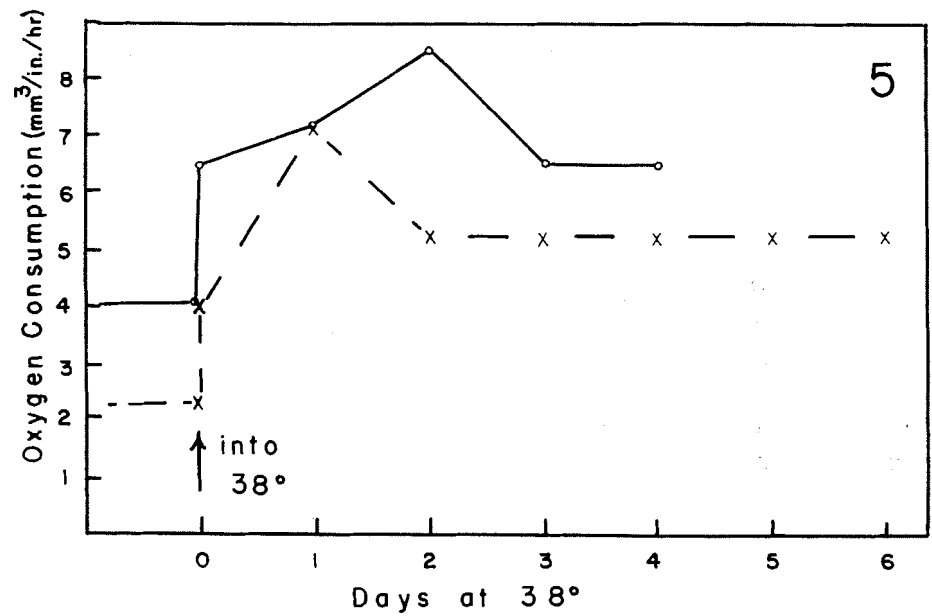
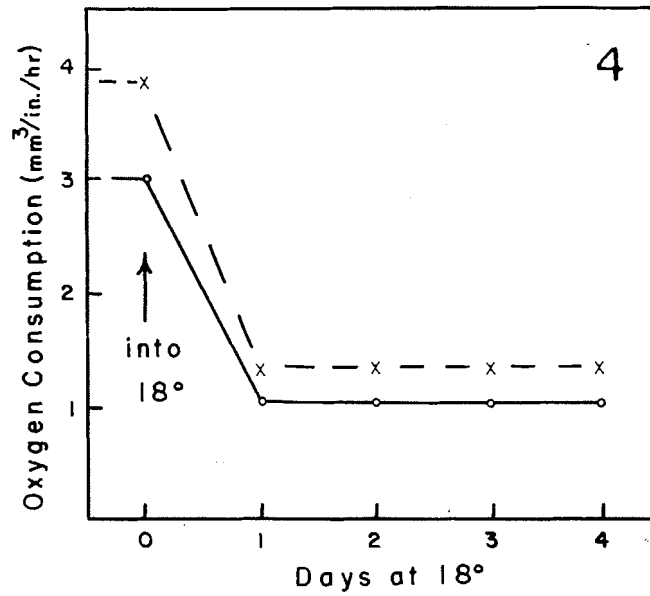


FIG. 4. Change in respiration of a sample of 10 male (broken line) and one of 10 female (solid line) 30° *T. confusum* during exposure to 18° C.

FIG. 5. Change in respiration of a sample of 10 male (broken line) and one of 10 female (solid line) 30° *T. confusum* during exposure to 38° C.

D. Weight

(i) Sex Differences

The wet weights of all samples of 30° control *T. confusum*, used to calculate the oxygen consumption per milligram, were recorded and the data analyzed to test for a possible difference in weight between the sexes.

A total of 60 samples (10 insects each) of males gave a mean weight of 20.01 mg. per sample. For females, a total of 55 samples gave a mean weight of 23.11 mg. per sample. The difference in weight is highly significant.

(ii) Respiration-Weight Regression

The five oxygen consumption readings at each temperature for both sexes of control 30° *T. confusum* are plotted against corresponding weights for the samples in Fig. 6. In some cases, for example in males at 34° C. and 44° C.,

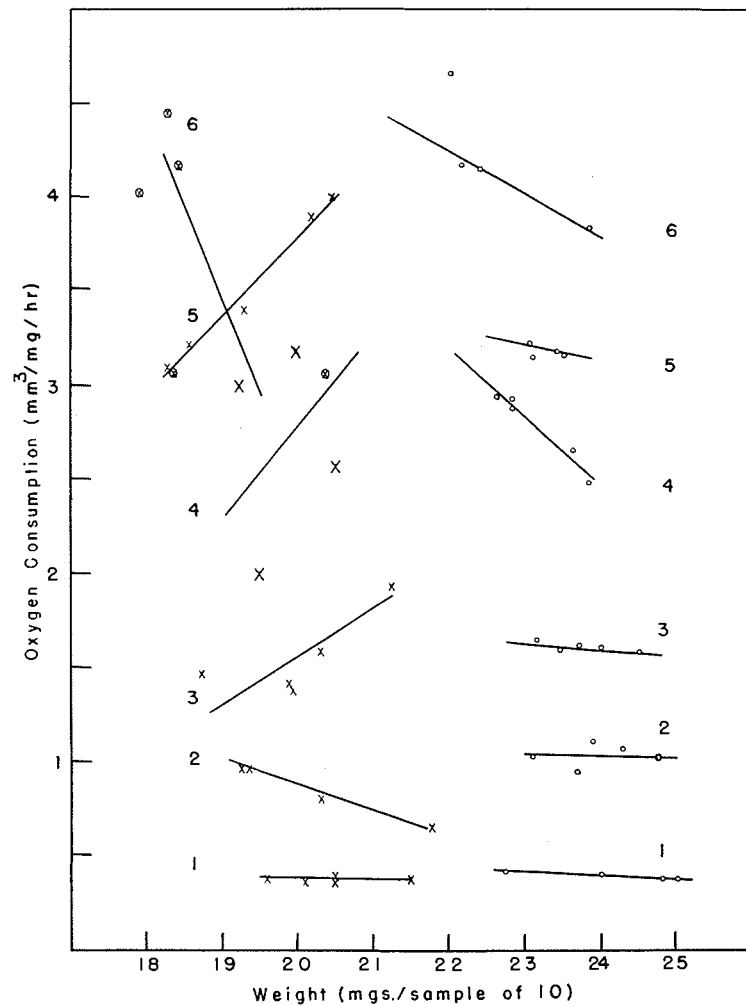


FIG. 6. Respiration-weight regression at different experimental temperatures for male (smaller weight) and female 30° *T. confusum*. 1, 10°; 2, 18°; 3, 26°; 4, 34°; 5, 40°; 6, 44°.

the scatter of the points is fairly large. Thus, the slope of the line indicated in these cases is not too reliable.

The sign of regression is essentially opposite in males and females at each temperature except 18° C. and the upper extreme. In females, increased weight is correlated with a lower oxygen consumption at each experimental temperature, while in males the opposite is true. In both sexes respiration is less size dependent at low temperatures. In females, there is a decreasing Q_{10} with increasing weight except between 34° C. and 40° C. Lighter females were more sensitive to temperature change. In males, there is an increasing Q_{10} with increasing weight between 18° C. and 26° C., 26° C. and 34° C., and between 26° C. and 40° C. Heavier males were more sensitive to temperature change.

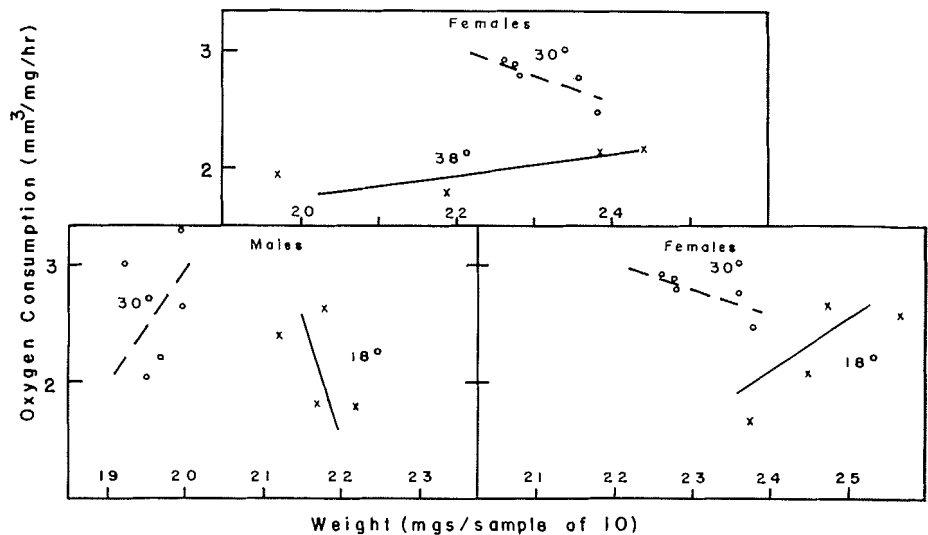


FIG. 7. Respiration-weight regression of 18° male and female, and 38° female *T. confusum* compared with that of 30° insects; measured at 34° C.

The effect of long-term maintenance of the insects at the nonoptimal temperatures on respiration-weight regression is shown in Fig. 7. The temperature 34° C. was chosen since data from the T-R curves could be used, and this temperature is close to the optimum. Prolonged exposure to the non-optimal temperatures resulted in a reversal of the regression sign. Lighter females and heavier males were affected most. This sensitivity difference among individuals of different weights is in agreement with the situation shown in Fig. 6.

(iii) *Weights of Nonoptimal-Temperature Groups*

Menusan (21) reported that the weight of adult *T. confusum* increased as the temperature of the environment decreased, with food moisture and relative humidity constant. The wet weights of both 18° and 38° insects in the present work are compared with values for 30° controls in Table VI. Weights were based upon samples of 10 insects.

TABLE VI
WEIGHTS OF SAMPLES OF 30° CONTROL, 18°, AND
38° *T. confusum*

Group	Sex	Weight, mg.	No. of samples	Sum of squares	Variance
30°	Males	20.01	60	75.62	1.28
30°	Females	23.11	55	137.79	2.55
18°	Males	21.36	22	8.56	0.41
18°	Females	24.58	28	14.18	0.53
38°	Females	22.18	25	34.94	1.46

The weight of 18° insects, males and females, is significantly higher, and that of 38° females significantly lower, than the weight of controls of the same sex.

(iv) *Water Content*

A test was made to determine whether the difference in weight between control and 18° insects was related to possible differences in the water content of the insects.

The wet weights of samples of 30° control insects, consisting of 34 males and 34 females, were determined. The insects were then placed in a vacuum desiccator at 60° C. for 2 days. The dry weight was then determined. Table VII gives the wet and dry weight of 30° and 18° insects in two tests.

Although the 18° insects possessed a wet weight greater than that of the controls, the dry weight of 18° insects was less than the dry weight of control insects. The greater weight of 18° insects originally found seems to have been due, therefore, to a greater water content compared with 30° controls. The relative increase in water was sufficiently great to mask the decreased dry weight in 18° insects. Table VII reveals that the greater wet weight of females compared with males is reflected in a greater dry weight in the former.

TABLE VII
WET AND DRY WEIGHTS (MG.) OF BOTH SEXES OF 30° CONTROL AND
18° ADAPTED INSECTS FROM TWO TESTS; $n=34$

		30°		18°		
		Test		Test		
		I	II	I	II	
Males	Wet	75.74	73.03	Wet	76.71	76.70
	Dry	39.80	36.87	Dry	33.97	33.22
	Water	35.94	36.16	Water	42.74	43.48
	% water	47.5	45.5	% water	55.7	56.7
Females	Wet	84.01	79.25	Wet	89.87	87.10
	Dry	46.83	40.73	Dry	38.82	37.84
	Water	37.18	38.52	Water	51.05	49.26
	% water	44.3	48.6	% water	56.8	56.6
Average % water, male and female		45.9	49.0		56.2	56.7

Discussion

A. Respiration

The fitted temperature-respiration curves for 30° males and females, when extrapolated, pass through the origin. It does not follow from this, however, that the respiration of the insects necessarily becomes zero at 0° C. The curve is described as being a good fit between the experimentally determined limits only. Respiration is maintained at a very reduced degree into the subzero temperatures. It has been shown that the insects may survive -3° C. for a short time.

Park (24) reported that female *T. confusum*, in his experiments, consumed significantly more oxygen than males per unit weight. His results were based upon probable error.

Saakyan (31) reported that the "breathing intensity" of *T. confusum* males is higher than that of females, especially in imagoes. No statistical analysis of the results was presented.

The present work has not shown a significant difference in oxygen consumption per unit weight between the sexes at each experimental temperature. There was no sex difference in the two constants of the T-R equations. There does exist, however, a *trend* in the direction of a higher oxygen consumption in females over the whole temperature range, in tentative agreement with Park. This is indicated by the higher value and confidence limits of *a* in both 30° females and 18° females compared with corresponding males. Female insects weighed more than males. On the basis of the "surface rule" (26) females might be expected to show less oxygen consumption per unit weight compared with males if the former were larger. However, there is no evidence that females were larger. The observed difference in respiration between the sexes may have been related to the sex difference itself, as originally suggested by Park, or to relatively more respiring tissue in females.

No definite reason can be given at this time for the differences in respiration per unit weight between lighter and heavier individuals of the same sex. However, some speculation on this point might be of value. Larger insects would possess proportionately less cuticle compared with body volume, as a result of a smaller relative surface area. In larger insects the *relative* decrease in "inactive" cuticle, compared with the volume of respiring tissue, would tend to increase the value obtained for oxygen consumption on a unit weight basis. This would aid in explaining the increased oxygen consumption in larger 30° males compared with smaller ones. The greater oxygen consumption would also be facilitated if the greater weight per se was related to a greater amount of more active tissue (for example, sperm).

In 30° females the lower oxygen consumption per unit weight in heavier individuals is in apparent agreement with the "surface rule", if "heaviness" was related to size. This trend toward decreased oxygen consumption would be opposed by the relative decrease in the amount of "inactive" cuticle. However, there is no evidence that "heaviness" was related to size. A greater mean weight in some females compared with others may reflect the weight of

eggs in the former. More eggs would mean a greater relative "inactive" weight, due to an increased amount of stored food, and would tend to lower the oxygen consumption per unit weight. Bodine (6) has reported that the greater weight in female *Dichromorpha* (Orth.) compared with males is due to the weight of eggs in the former.

To summarize these points, the trend toward a greater oxygen consumption in females, compared with males, may be related to a greater relative amount of respiring tissue in the former. The decreased oxygen consumption per unit weight in heavier females, compared with lighter ones, may have been related to a greater abundance of eggs in the former. The greater respiration in heavier males, compared with lighter ones, may have been related to relatively more respiring tissue in the former.

The depression of both the high- and low-temperature group respiration curves is in agreement with some of the results obtained by Agrell (1) for *Anagasta* (formerly *Ephesia*) *kühniella* and *Forficula auricularia*. Agrell did not establish a temperature-respiration curve over a range of temperatures in his acclimatized insects.

The constant b in the respiration curve reflects the degree of curvature of the T-R line over the total temperature range and represents the slope of the log-log line. The increase in b and the decrease in a in the 18° and 38° group curves compared with 30° controls is reflected in a counterclockwise "rotation" of the former about the region of maximum oxygen consumption, with respect to the curve for 30° controls. This situation is illustrated in the semilogarithmic curves for females in Fig. 2.

Rao and Bullock (27) have reported that the Q_{10} is lower in cold adapted poikilotherms than in those adapted to high temperatures, the constant varying with thermal adaptation. Scholander *et al.* (32), however, stated that the Q_{10} does not vary significantly with cold adaptation. Rao and Bullock themselves did not consider a change in Q_{10} in itself to be evidence for thermal adaptation. Bělehrádek (4) reported that the thermal coefficient rises in a number of cases with adaptation to a higher temperature. In the present work, the Q_{10} of the 18° adapted insects was greater at any given temperature than that of controls at the same temperature. The same also applies to the 38° insects.

Analysis has shown no significant difference in a or b between the 18° and 38° females. This apparent similarity may well be only superficial and is probably not due to similar metabolic states. The first evidence in favor of this is found in the mortality at extreme temperatures. It has been demonstrated that the survival of 38° insects at 40° C. was less than that of 18° insects at this temperature. Thus, long-term exposure to 38° C. did not enhance the ability of the insects to survive high temperatures. The 18° insects showed increased cold hardiness and slightly increased heat hardiness as well. Secondly, the 18° insects appeared, when stimulated, to be as vigorous as the 30° controls, or perhaps even more so. It was much more difficult to keep the former confined in a glass tray at room temperature, compared with 30° controls. The 38° insects, however, were very slow moving at all times. Some

exhibited poor motor coordination after some time at 38° C. Finally, no respiratory compensation was exhibited in the 18° insects. The position of the 38° curve can be interpreted in either of two possible ways. Either the insects exhibited respiratory compensation at the high temperature, or their metabolism was depressed by adverse effects of hyperthermy. It does not seem probable that this species would show respiratory compensation at high temperatures without also exhibiting it at low temperatures.

When the insects are first transferred to 18° C. no respiratory compensation is evidenced; a "passive" lowering of the respiration occurs. This corresponds to the lower portion of the control T-R curve. When transferred to 38° C., however, a respiratory increase followed by depression occurs in both sexes. No definite reason for the depression can be given at this time. The 38° respiration depression may possibly have been the result of, first, a positive accelerating effect on respiration due to the higher temperature, followed by a deleterious, retarding effect.

An alternative explanation for the increased respiration and subsequent depression involves the possible differential effect of the high temperature on different tissues. The slow, prolonged increase in respiration following the initial rise may itself indicate detrimental effects of heat on the enzymatic processes. When the point of maximum respiration had been reached a particular group of tissues more sensitive to high temperatures than the rest may have succumbed completely. This would reduce the general respiration per unit weight to a lower level. The upper lethal temperature range for this species is very close to 38° C. (23).

Since both the sex and the ratio of active to inactive weight seem to have influenced values obtained for respiration per unit weight in the present work, these factors should at least be considered in all work of this kind. In situations wherein there may exist differences in the ratio of active to inactive weight between two temperature groups these considerations are especially applicable. An example is seen in comparing the respiration per unit weight of some marine invertebrates from different environmental temperatures utilizing dissolved carbonate. The difference in temperature would result in a difference in the availability of dissolved carbonate, as a result of shifting in the carbon-equilibrium equation. Hence, oxygen consumption values based upon a unit of weight would tend to give higher values in organisms from colder water due merely to a *relatively* smaller amount of inactive tissue present in, for example, the exoskeleton. This shift in the oxygen consumption values would be in the same direction as that previously reported in respiratory compensation studies (7).

B. Weight Changes

The increased weight in 18° insects was the result of an increased water content, possibly as a result of a decreased saturation deficiency at 18°. These results do not agree with the conclusions of Menusan (21) that weight changes at lower temperatures at the same relative humidity in *T. confusum* were not due to differences in the water content of the insects.

Undoubtedly, the increased water content, together with a decreased dry weight in 18° insects served to reduce the values for oxygen consumption on a unit weight basis. On this basis alone, however, the 18° curve would be expected to be depressed more at higher temperatures and less at the lower ones. This is not the case. Apparently some other factor, in addition to artifact, seems to have been responsible for depression of the 18° curve as proposed by Agrell (1). This additional factor seems to have been reflected in the increased curvature (*b*) in the 18° curve.

The differential effect of weight on the respiration within a given temperature group would not have substantially influenced the T-R curves. The latter were determined on the basis of the mean respiration of five samples of insects at each temperature.

C. *Survival at Extreme Temperatures*

The sex difference in survival ability at -3° C. in 30° control insects was not indicated at 40° C. It is difficult to be certain as to whether this differential survival ability at the low temperature was due primarily to factors related directly to sex itself or to the factor responsible for the difference in weight between the two sexes. Lighter females were more sensitive to temperature change, while heavier males were the more sensitive. In other words, "lightness" in males may have more survival value and in females "heaviness" may have more survival value at the lower temperatures. A more direct factor may have been chiefly responsible for the mortality at -3° C. This factor was more critical in females than in males.

Long-term exposure of *T. confusum* to 18° increased heat resistance as well as cold resistance, as evidenced by a greater LD₅₀ at 40° C. in the 18° insects. The acclimatizing temperature of 18° C. is far from the physiological optimum of the insects (14). The temperature factor in the environmental resistance should increase away from the physiological optimum. It is possible that an increase in environmental resistance in the form of a reduction of the temperature to 18° C. was counteracted by an increase in thermal resistance in the insect. This increased resistance, as well as enhancing the survival ability at low temperatures, may also have served in some manner to increase the resistance to high temperatures.

The greater water content in 18° insects, together with the increased survival ability at both -3° C. and +40° C. constitutes some evidence against theories concerning a positive relationship between desiccation and thermal resistance (2, 6, 25, 28). Nevertheless, the possibility of a positive relationship between protoplasmic viscosity and thermal resistance cannot be excluded. Heilbrunn (15) has shown that eggs of the sea urchin *Arbacia* in sea water diluted with distilled water exhibit increased protoplasmic viscosity through coagulation when the dilution is 60-70% distilled water. On the basis of arguments put forth by Bělehrádek (5) the increased coefficient *b* in the T-R curves of 18° insects seems to constitute evidence for increased protoplasmic viscosity.

The reduction in the viability of *T. confusum* exposed to 38° C. for a prolonged period may have resulted, at least partially, from excessive dehydration. The importance of the time factor in heat death has been emphasized by Orr (22). In the present work, 4.02 days at 40° C. has been shown to be the time necessary to kill 50% of control insects, at a relative humidity of 75%. Impairment, and possibly death, at 38° C. under the same conditions of humidity would mean extension of the time factor. The latter might be in the vicinity of 6 or 7 months, the time during which the present insects were subjected to 38° C.

D. The Temperature Coefficient

Most reports in the past have shown that Q_{10} decreases with rising temperature in many biological reactions (17). The present work has supported this. This variation in Q_{10} introduces the variable *temperature range* into comparisons of Q_{10} between organisms.

In cases where acclimatization results in horizontal shifting of the temperature-metabolism curve (7), comparisons on the basis of Q_{10} are particularly confusing. A given temperature range applies to different relative portions of the curve. For example, a given T-R curve may be shifted to the left when a poikilotherm exhibits respiratory adaptation to cold. At any given temperature the respiration of the cold adapted form is higher than that of the organism adapted to the higher temperature. Therefore, if a given temperature range along the x axis is projected upward through the T-R curves, it will cut off a section on the cold-adapted curve which is much nearer the latter's maximum thermal limit than the section cut off on the warmer-environment curve by the same projection. Hence, since the Q_{10} normally decreases upward along the T-R curve, one might tend to conclude that cold adaptation, in addition to resulting in increased respiration, shows a lower Q_{10} if only this particular temperature range is considered.

The critical thermal increment (μ) in the Arrhenius equation represents another source for confusion. Observation of the semilogarithmic plots *without* the lines drawn through them frequently gives more an impression of a continuous curve, rather than two or more straight lines. The exponential Arrhenius equation represents an assumption that the semilogarithmic line would be straight. Some excellent examples of this situation have been given by Bělehrádek (5).

Bělehrádek has stated that the straight line proposed originally by Krogh, and the Arrhenius equation, are only special cases of a more general situation described by the log-log type equation. The constant b in the present work, which might be referred to as the "thermal-sensitivity coefficient", might be useful in comparing T-R curves of the log-log type in various experimental groups. The constant takes into account the degree of response of the organism to temperature change over the total effective temperature range, and does not vary with the temperature range chosen.

Conclusions and Summary

1. The present work has shown no significant difference in the oxygen consumption per unit weight between male and female adult *T. confusum*. However, there is a trend in the direction of a higher respiration rate in females.

2. Prolonged exposure to 18° C. resulted in a lowering of the T-R curve, as did prolonged exposure to 38° C. In both cases the curves exhibited increased curvature and tended to approach that for control 30° insects near the upper thermal limit.

3. Acclimatization was evidenced in the 18° insects by increased survival ability at -3° C. and not by respiratory compensation. There was no evidence for acclimatization in the 38° insects.

4. In control 30° *T. confusum*, males survived exposure to -3° C. to a greater degree than females. This sex difference in survival at the low temperature was eliminated by prolonged exposure to 18° C. Lighter females and heavier males were more sensitive to temperature changes. There was a corresponding sex difference in the respiration-weight relationship.

5. Female *T. confusum* possessed a greater wet and dry weight than males. Changes in the amount of respiring tissue in proportion to the amount of water were probably reflected in depression of the T-R curve of 18° insects. Some additional depressing factor was probably also involved.

6. Both sex and the ratio of active to inactive weight in *T. confusum* may influence the respiration-weight regression and also the numerical value of the oxygen consumption per unit weight. These factors should be taken into account when dealing with any problem of this kind.

7. The temperature-respiration curves in the present work were adequately described by a double-logarithmic type equation within the limits of the experiments. The constant *b* in the equation, or "thermal-sensitivity coefficient", might be more valuable in comparing temperature-metabolism curves of various temperature groups than exponential constants based upon semi-logarithmic interpretations. The former is independent of the temperature range chosen.

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